IN BRIEF

Sibling Rivalry: How Two Proteins from a Common Ancestor Play Opposing Roles in Fatty Acid Biosynthesis

All plant cells contain lipids. These highly diverse hydrophobic compounds make up the bulk of the membrane and function in energy storage, protection against dehydration and pathogen attack, photosynthesis, and other vital processes. In addition, plant lipids, especially seed oils, represent an important source of food and renewable energy whose biosynthesis can potentially be manipulated. Although most lipid biosynthetic pathways in plants have been elucidated (Ohlrogge and Browse, 1995), little is known about how these pathways are regulated, impeding our ability to engineer plants with increased oil content. Acyl chains for lipids are primarily produced in plastids via the de novo fatty acid synthesis (FAS) pathway. The first committed step of this pathway is catalyzed by acetyl-CoA carboxylase (ACCase), making this enzyme an attractive focus of study (reviewed in Salie and Thelen, 2016). In eudicots and nongraminaceous monocots, ACCase is present in a heteromeric (hetACCase) form, comprising four distinct subunits: biotin carboxylase, biotin carboxyl carrier protein (BCCP), and α- and β-carboxyltransferase. While these subunits are known to form subcomplexes, hetACCase is quite difficult to purify using standard procedures, hampering efforts to explore the structure and function of this crucial enzyme. Moreover, while we know that hetACCase activity is regulated by exposure to light, the signaling protein PII, feedback inhibition by acyl-acyl carrier protein (the final product of de novo FAS), and redox chemistry, these mechanisms don’t account for all of the known features of this enzyme.

Salie et al. (2016) recently uncovered an intriguing new mechanism for the biochemical regulation of hetACCase. The study began with a search for interacting partners of hetACCase via in vivo coimmunoprecipitation of chloroplast proteins from Arabidopsis thaliana seedlings using subunit-specific antibodies. Analysis of the immunoprecipitated proteins using liquid chromatography-tandem mass spectrometry and bioinformatics showed that they comprise a novel family of proteins of unknown function with a putative biotin/lipoyl attachment domain-containing domain. These proteins, which they designated BADC (biotin/lipoyl attachment domain-containing) proteins, were confirmed to interact with the BCCP subunit of hetACCase by yeast two-hybrid assays and coexpression analysis in Escherichia coli. Interestingly, while BADC proteins closely resemble BCCP, they are not biotinylated due to the presence of a mutated biotinylation motif. Phylogenetic analysis suggested that BADC proteins are conserved in land plants and may have originated by duplication of the BCCP gene in red algae, followed by a loss-of-function mutation.

Since BADC proteins resemble BCCP but lack a functional biotinylation motif, the authors speculated that BADCs negatively regulate hetACCase activity. Indeed, a temperature-sensitive BCCP E. coli mutant was rescued by transformation with wild-type BCCP, but this complementation was suppressed by the presence of Arabidopsis BADC3. Enzyme activity assays using Arabidopsis leaf extracts confirmed that the BADCs negatively affect hetACCase activity (see figure). RT-qPCR analysis indicated that in the light, genes encoding hetACCase subunits are upregulated in developing siliques, whereas BADC genes are downregulated. Finally, RNAi silencing of BADC1 using a seed-specific promoter increased the oil content in Arabidopsis seeds, reinforcing its role as a negative regulator of de novo FAS.

The authors proposed that BADCs negatively regulate hetACCase activity by competing with BCCP for binding to other hetACCase subunits. In this model, the delicate balance between BADCs and BCCP acts as a molecular rheostat to fine-tune the activity of hetACCase. Sibling rivalry is not always a bad thing: Perhaps the opposing actions of these related proteins could be exploited to engineer seeds with increased oil content and as a launching point to further explore the regulation of FAS in plants.

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REFERENCES


BADCs reduce ACCase activity in Arabidopsis. ACCase activity was monitored in leaf extracts with increasing concentrations of recombinant BADC1 (0 to 15 μM; 0 μM controls were normalized to 1). Error bars denote ± (n = 4), (Figure adapted by M.J. Salie from Salie et al. [2016], Figure 7C.)
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